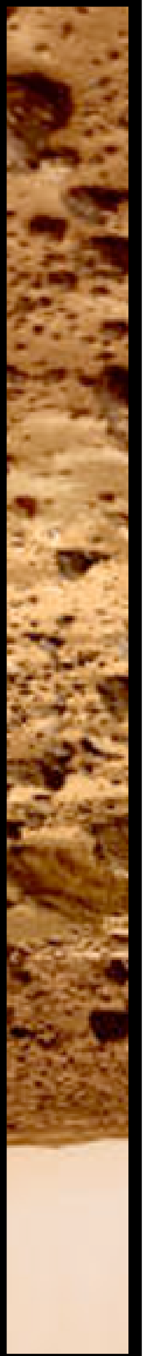
AstroBioLab: A Mobile *in situ* Subsurface Biotic Detector and Soil Reactivity Analytical Laboratory



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The AstroBioLab Concept

AstroBioLab is a mobile laboratory that uses a complementary suite of *in situ* instruments that focus on state-of-the-art organic compound detection coupled with determinations of the oxidation chemistry.

- The AstroBioLab is designed around the Mars Organic Detector (MOD) instrument, interfaced with a micro-chip capillary electrophoresis (μCE) system for enhanced analytical capacity, along with Mars Oxidant Instrument (MOI) that provides a complementary set of oxidant data.

- The target compounds are amino acids and PAHs, the major components of bacterial cells and carbonaceous meteorites.

- AstroBioLab field studies in the Mojave and Atacama deserts under flight–like conditions will demonstrate the feasibility of using this instrumentation to explore for signs of organic compounds of possible biological origin during future landed missions to Mars.

AstroBioLab: Technical Aspects

Simple Mechanically Robust Sub-Systems
- ✓ pBN crucible technology with high thermal efficiency and self cleaning ability to remove traces levels of contaminants
- ✓ Electronics designed for Martian ambient survivability (-145 to +100 °C)

Optical detection developed from single molecule detection systems

State-of-the-art microfluidic systems for chiral amino acid analysis
- ✓ Self contained fluidics control for sample acquisition from mod cold finger
- ✓ Chemical storage designed for long-term dormancy. buffers and reagents flown dry, activated as needed
- ✓ Small mCE analysis volumes used for statistics through replicate determinations

Liquid extraction system designed for rapid throughput, low power consumption, with broadest chemical extraction ability

MOI designed as low overhead minimal data stream experiment with periodic measurements over days/weeks exposure

AstroBioLab: Scientific Investigation

Search for the presence of key biomolecules in Martian-like environments with a sensitivity of 100x to 1000x better than the Viking

- ✓ Targets PAHs because of they are a possible indicator of abiotic chemistry and have excellent fluorophore detection chemistry
- ✓ Targets amino acids because of possible biogenic and abiotic origins an have excellent fluorophore detection chemistry
- ✓ Add amino acid chiral resolution and compound identification to resolve biotic or abiotic origin
- ✓ Observed compositions can suggest mechanisms of formation

Complement basic sublimation sample processing with high pressure sub-critical liquid extraction system

Couple *in situ* measurement of oxidation chemistry for direct assessment of oxidation processes effecting organic abundance

AstroBioLab: Measurement Objectives

MOD/μCE

✓ **Measure multiple samples (limited by sample acquisition system) to determine presence (in 100 to 500 mg samples) of amino acids, amines or PAHs at femtomoles (10^{-15} moles) sensitivity.**

✓ **Determine amino acid composition of extracted compounds with a sensitivity of femtomoles or better.**

✓ **Determine amino acid D/L chirality ratio with a precision of ± a few %.**

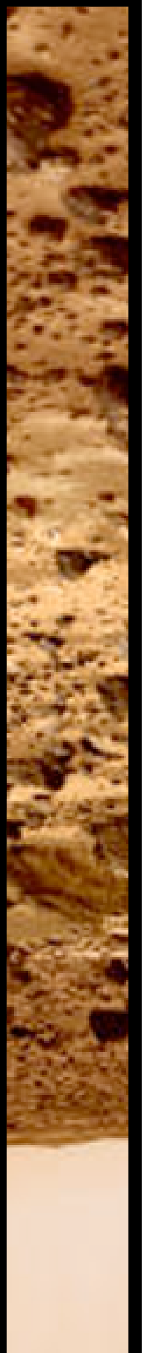
MOI

✓ **Determine reaction kinetics of oxidation for at least 6 reference reactants of at least 10 sample with measured organic compositions.**

✓ **Determine impact of controlled water levels in replicate oxidation experiments.**

AstroBioLab Goals

The overall goal of the AstroBioLab based analyses is to demonstrate the feasibility of using similar instrumentation to search for organic compounds directly in the surface of Mars and if compounds are detected assess their origin



Why is the detection of organic compounds on Mars important?

- Organic compounds should be present because of the infal and long-term accumulation of of carbonaceous chondrite-like material on the Martian surface
- Organic compounds could have been produced by prebioti processes early in the history of Mars
- Organic compounds derived from either extinct or extant life could be present
- Characterizing organic carbon on Mars during future Mars missions is listed as one of the highest priorities in recommendations made by MEPAG
- The determination of the nature and inventory of organic carbon compounds will be one the main areas of focus for the Mars Science Laboratory (MSL)

The Infall of Carbonaceous Chondrite-like Material Could Have Delivered Organic Compounds to Mars

Murchison Meteorite
Australia, 1969

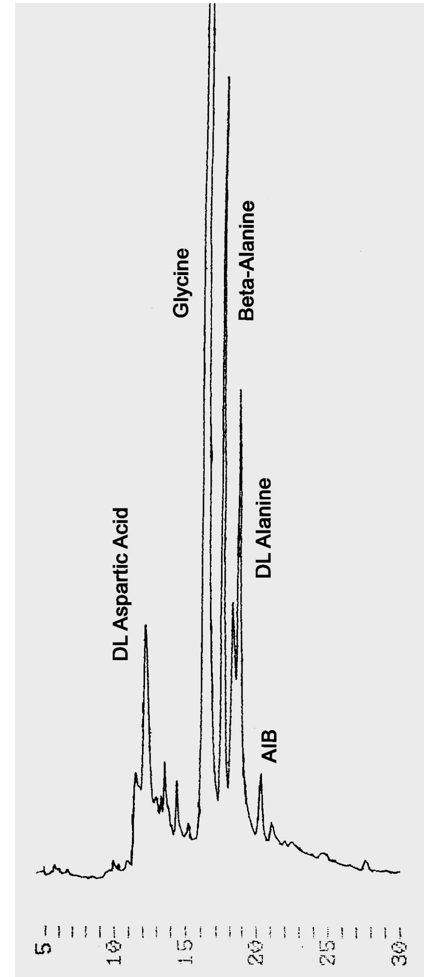
A summary of the abundances of soluble organic compounds in CI and CM chondrites.

Compound Class		Concentration (ppm)
Amino Acids*		
	CM meteorites	17-60
	CI meteorites	~5
Aliphatic hydrocarbons		>35
Aromatic hydrocarbons		3300
Fullerenes		>100
Carboxylic acids		>300
Hydroxycarboxylic acids		15
Dicarboxylic & Hydroxydicarboxylic acids		14
Purines & Pyrimidines		1.3
Basic N-heterocycles		7
Amines		8
Amides	linear	>70
	cyclic	>2
Alcohols		11
Aldehydes & Ketones		27
Sulphonic acids		70
Phosphonic acids		2

Botta and Bada, *Surveys of Geophysics* <u>23</u> 411–467 2002

* Many of the detected amino acids do not occur naturally on Earth and are unique to meteorites.

Miller-Urey Synthesis on Mars?



HPLC analysis of spark discharge mixture

When searching for evidence of life what molecules do we look for?

"The expected commonality of chemistry in life's processes assists in life detection because it predicts that terrestrial type of biochemicals are useful targets for analysis even in an extraterrestrial setting." N. R. Pace, The universal nature of biochemistry, *Proc. Natl. Acad. Sci. USA* 98, 805-808 (2001)

One note of caution -- although the core classes of molecules (amino acids, nucleobases, etc.) may be similar to terrestrial life, their overall structural makeup could be different. Although searches should be focused on key molecules, they should be general enough to detect molecules that are not necessarily the same as those used in terrestrial biology.

The molecules of life

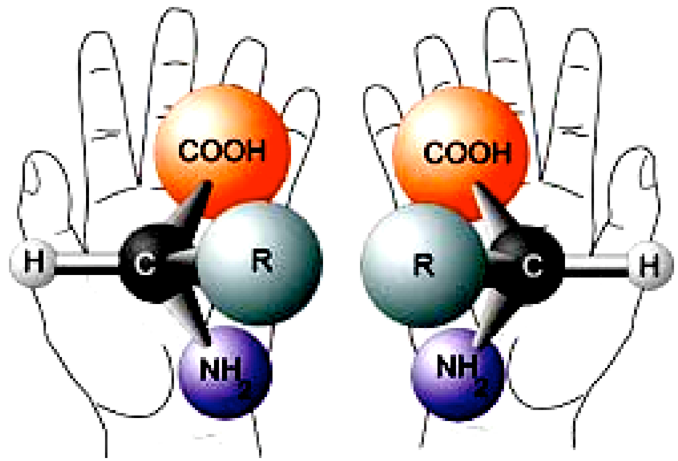
On a weight bases, amino acids are the most abundar single class of molecules in a typical bacterial cell. The next most abundant are the nucleobases (~1/10 as abundan associated with DNA and RNA.

Dry weight composition of E. coli. (g/cell)

protein	$1.6x10^{-13}$
RNA	$6x10^{-14}$
DNA	$0.9x10^{-14}$
lipids	$2.6x10^{-14}$
soluble fraction	$0.8x10^{-14}$
other	$2.4x10^{-14}$
Total	$2.8x10^{-13}$

From T. D. Brock, D. W. Smith and M. T. Madigan, Biology of Microorganisms, Prentice Hall, 1984

Amino acid chirality (handedness) can be used to evaluate abiotic vs biotic origins



L-amino acid D-amino acid

On Earth, only L-amino acids are encoded into proteins. In carbonaceous meteorites, both L- and D-amino acids are present in equal (or nearly equal) amounts. Life elsewhere could be based on either L- or D-amino acids.

What Viking May Have Missed

- It was claimed that the GC/MS instrument did not detect organics above part per billion (ppb) level.
- However, the detection limit for amino acids is now known to have been in the 10s of ppm range.
- A bacterial cell has a dry weight of 10^{-13} grams.
- Amino acids are the major organic component of cells.
- At ppm level, amino acids from $\sim10^{7}$ cells per gram of Martian soil would not have been detected!
- Thus, Viking did not necessarily rule out the possibility of Martian biology!

Modern day GCMS systems have better sensitivity than Viking for some compounds <u>but not amino acids</u>.

Glavin, D. P. et al., "Detecting pyrolysis products from bacteria on Mars" ***Earth. Planet. Sci. Letts.*** **185, 1-5 (2001)**

Mars Organic Detector (MOD)

- Compared to total organic carbon, Raman spectrometry or GCMS based measurements, MOD directly detects key organic compounds at very low concentration levels.

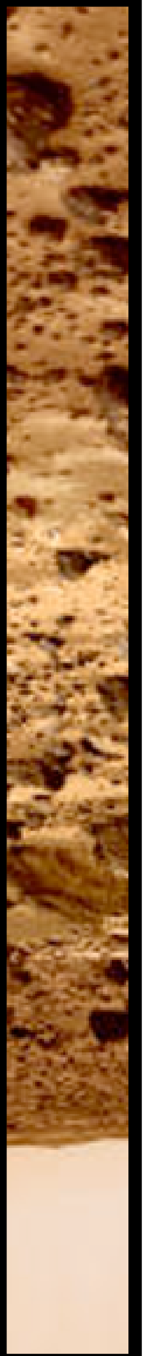
- The targeted organic compounds, amino acids and PAHs, are important in assessing the prebiotic and biotic potential on Mars and <u>other solar system bodies</u>.

- The MOD design is well advanced with prototype development funded by MIDP, PIDIP, ASTID and ASTEP grants. A version of the instrument was selected for the HEDS 03 lander instrument package and is on the short list of instruments on ESA ExoMars mission.

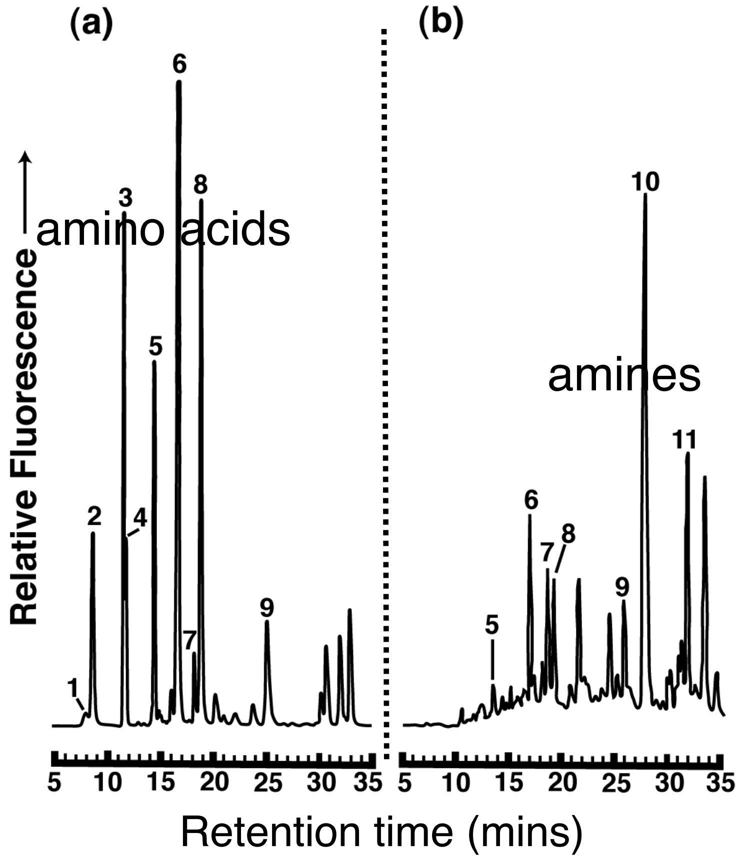
The Mars Organic Detector (MOD) Instrument

- Allows for the *in situ* detection of organic compounds directly on the surface of Mars and other planetary bodies.
- Uses sublimation at Mars ambient pressures and temperatures to release organic components of retrieved samples.
- Highly sensitive fluorescent detection is used to access the presence or absence of target organic compounds.
- MOD detects amino acids and PAHs at sub-ppb sensitivity.
- By comparing the fluorescent signal of the fluroescamine coated and uncoated regions of the MOD detector disk the relative amounts of PAHs and amino acids can be determined.
- MOD in interfaced with m-chip based capillary electrophoresis for identification and chiral resolution of any detected amino acids.

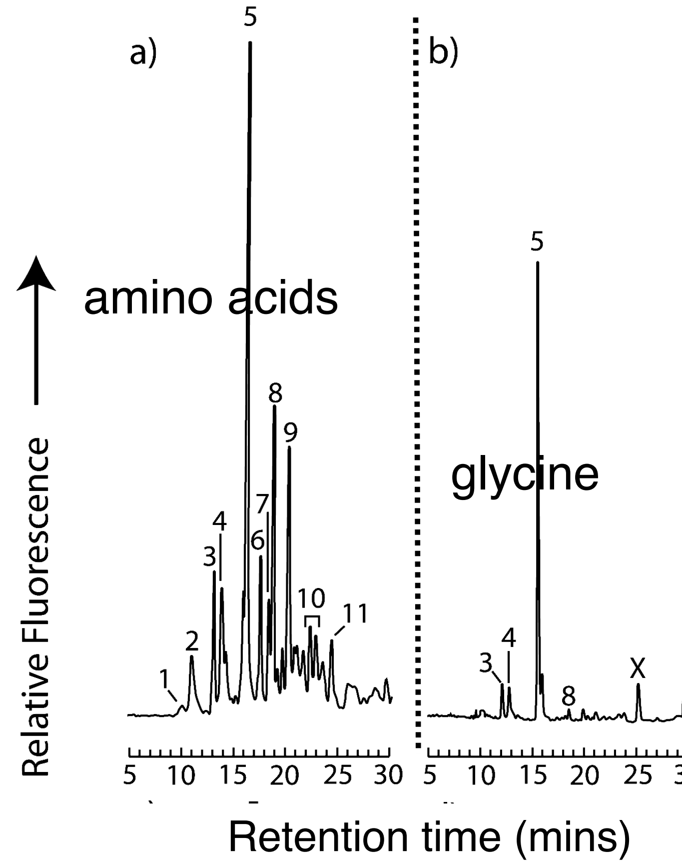
HPLC analyses of 6 M HCl hydrolyzed (a) and sublimed (b) samples



Palagonite + *E. coli* cells



Murchison meteorite

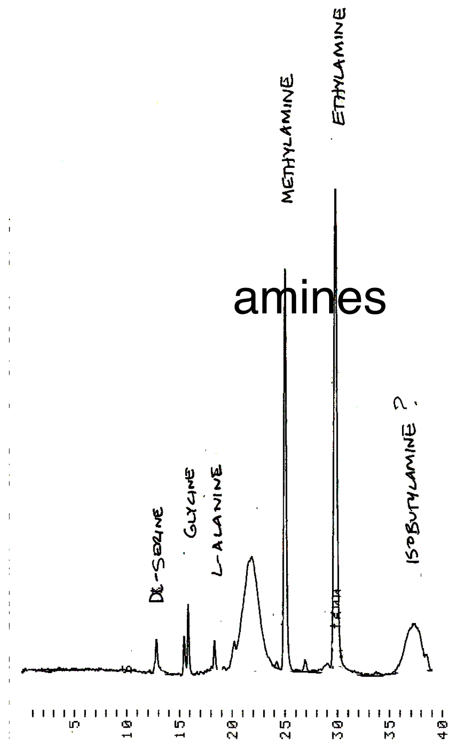


The detection of amines indicates bound amino acids are present and sample should first be extracted/hydrolyzed before sublimation

Hydrolysis of Bound Amino Acids Prior to Sublimation

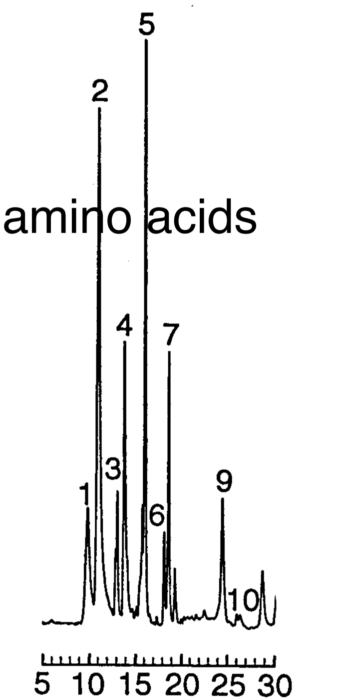
Results using a carbonate fossil sample (HPLC analyse

Direct sublimation



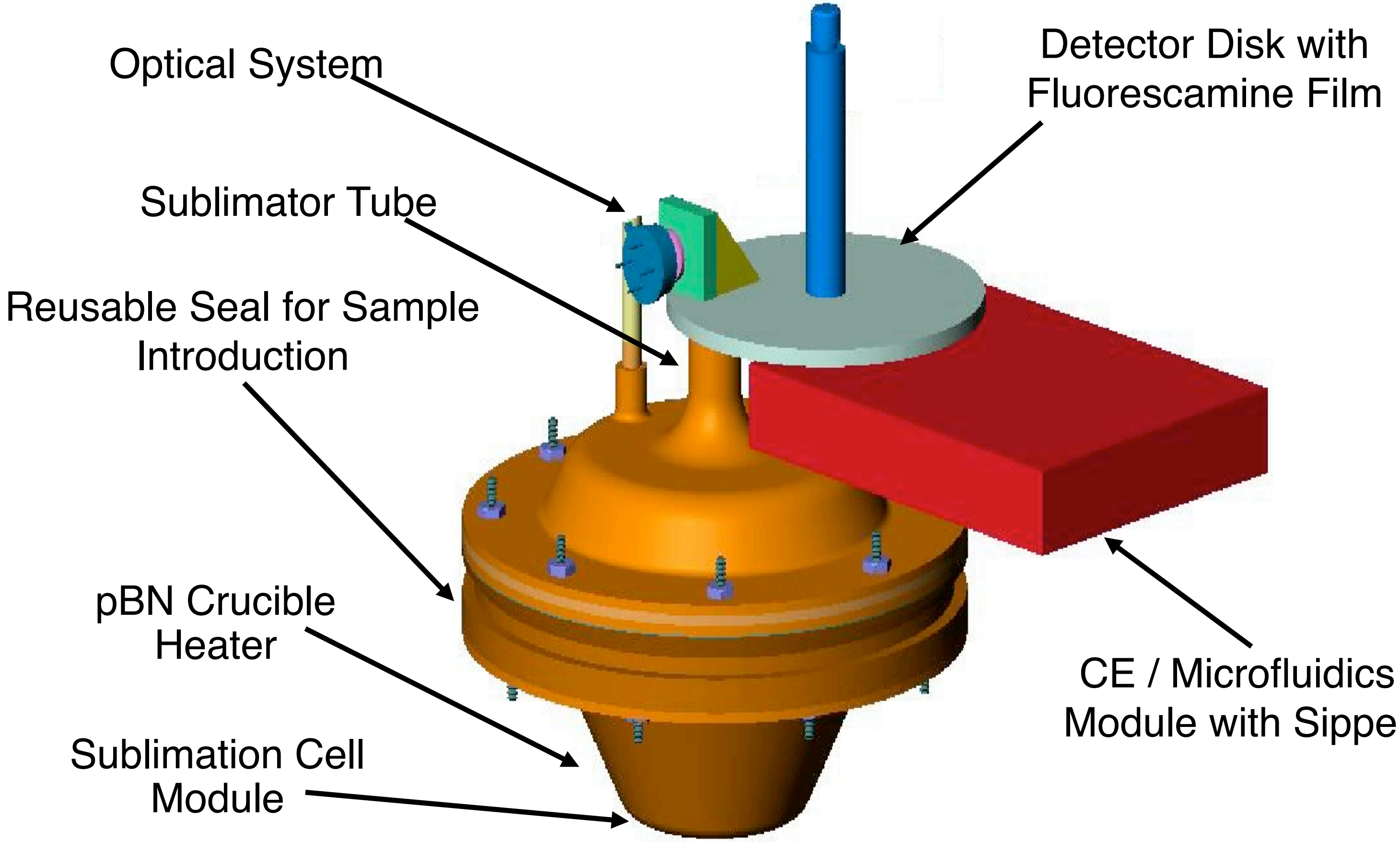
Retention time (mins)

Hydrolysis followed by sublimation



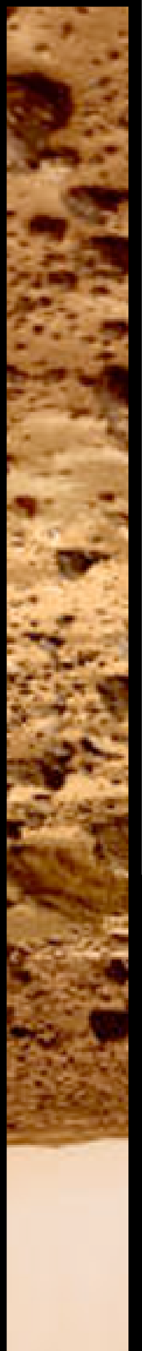
Retention time (mins)

Design of an integrated MOD/μCE system



Specifications:

Mass: ~2 kg
Power: 24 watts during experiment
Size: 145 mm (width), 193 mm (length), 112 mm (height)
Analyzes samples of crushed drill cores or soils collected by a drill or a scoop



Astrobiolab Operational Sequence

SAMPLE FROM ACQUISITION SYSTEM

↓

BASIC MOD INSTRUMENT

↙ ↘

No fluorescent signal implies the absence of target compounds or they are below detection limits

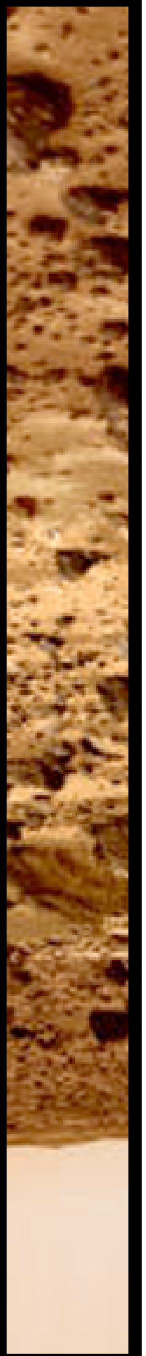
Fluorescent signal detected implying that target molecules are present in the sample

↓

Comparison of fluorescamine coated and un-coated areas of the detector disk can tell whether PAHs or amino acids/amines are present

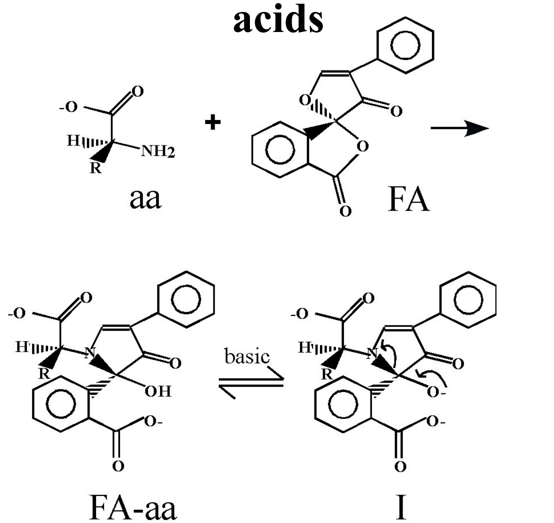
←

If amino acids/amines are detected μCE system is used to characterize the components. If amines are detected the sample is first extracted and then sublimed and analyzed

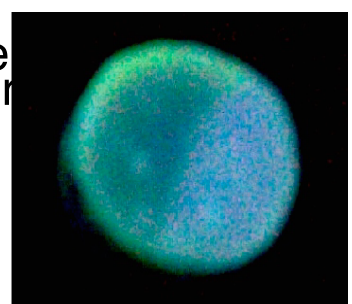


Fluorescent Detection Followed by Amino Acid Composition and Chirality Analyses

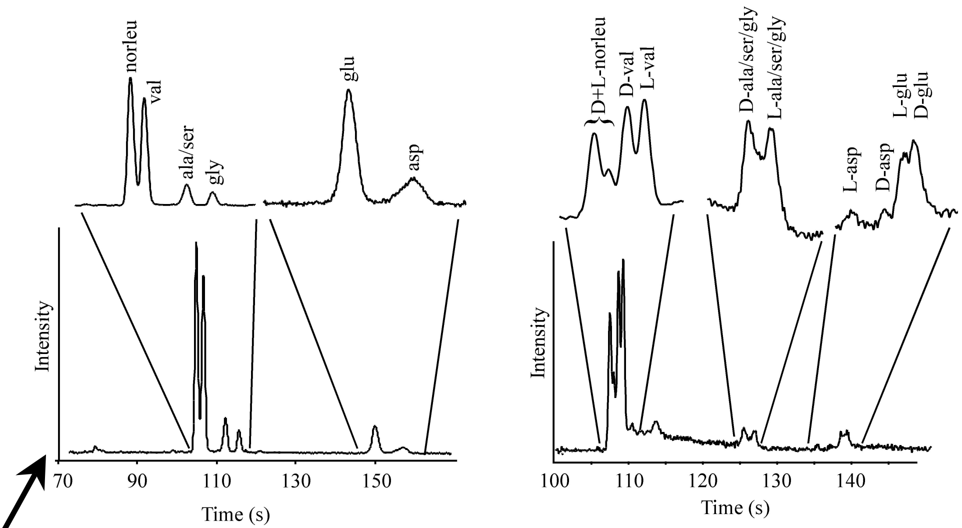
Reaction of Fluorescamine(FA) with amino acids



Fluorescence derivative indicates amino acids or amines are present



Amino Acid Composition **Amino Acid Chirality**



mCE analysis, **A. Skelley and R. A. Mathies,** ***J. Chromatography. A*** **(2003)**

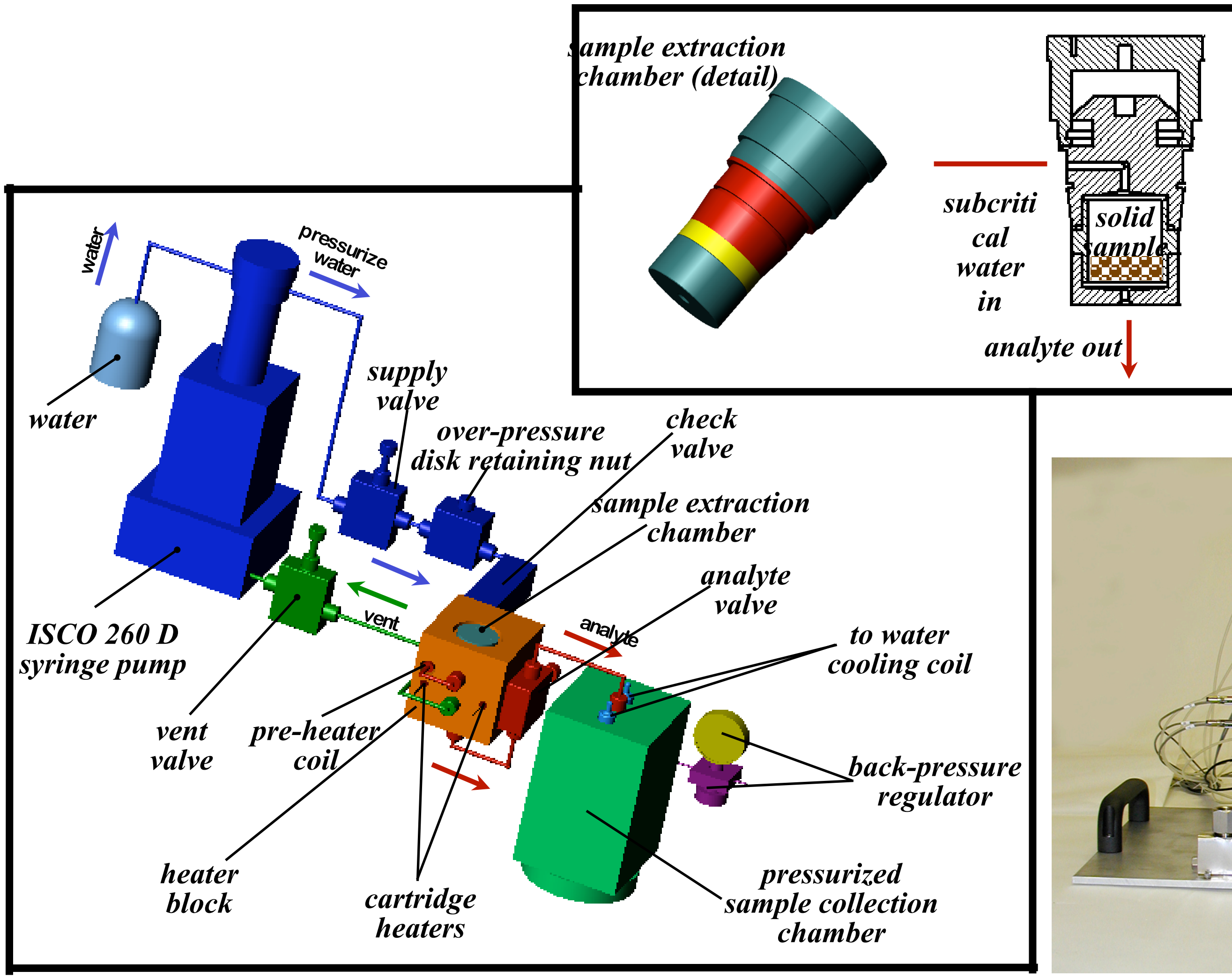
Sub-Critical Water Hydrolysis if Amines are Detected

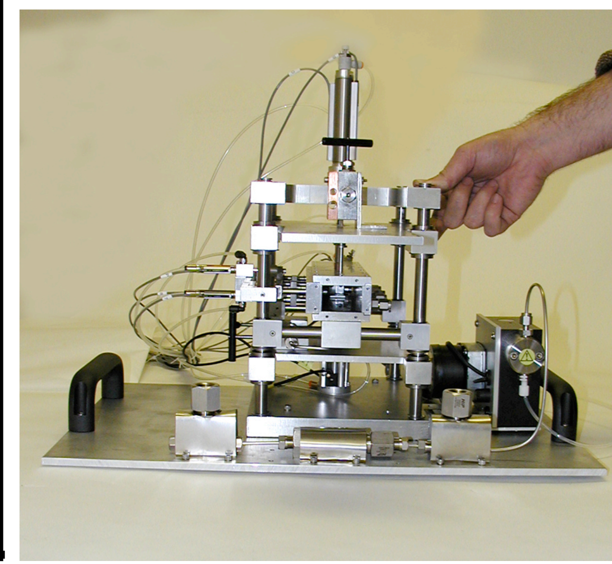
Below the critical point of water (647 K, 22.1 Mpa) hydrolysis is the dominant reaction and can be used to hydrolyze bound amino acids. There is a critical interplay between temperature
and racemization/decomposition and the conditions thus be optimized. Other solvents such as propanol may also be suitable.

Effect of temperature on D-leucine

T (°C)	Time (mins)	L/D	Degradation
	0	0.0000	-
100	10	0.0076	5.30%
150	10	0.0044	8.40%
200	10	0.3424	10.01%
243.2	10	0.9326	12.77%

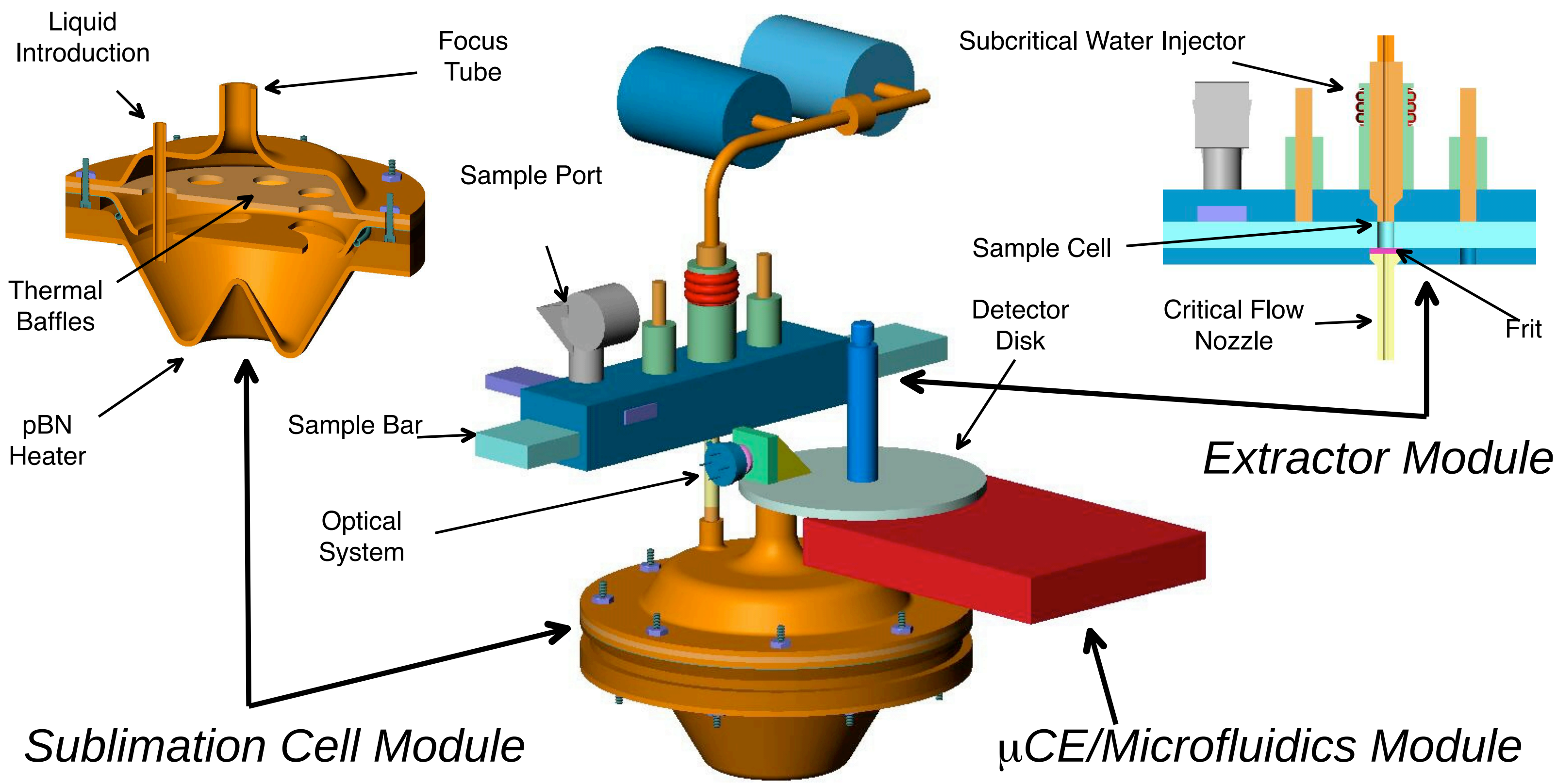
Sub-Critical Water Extraction



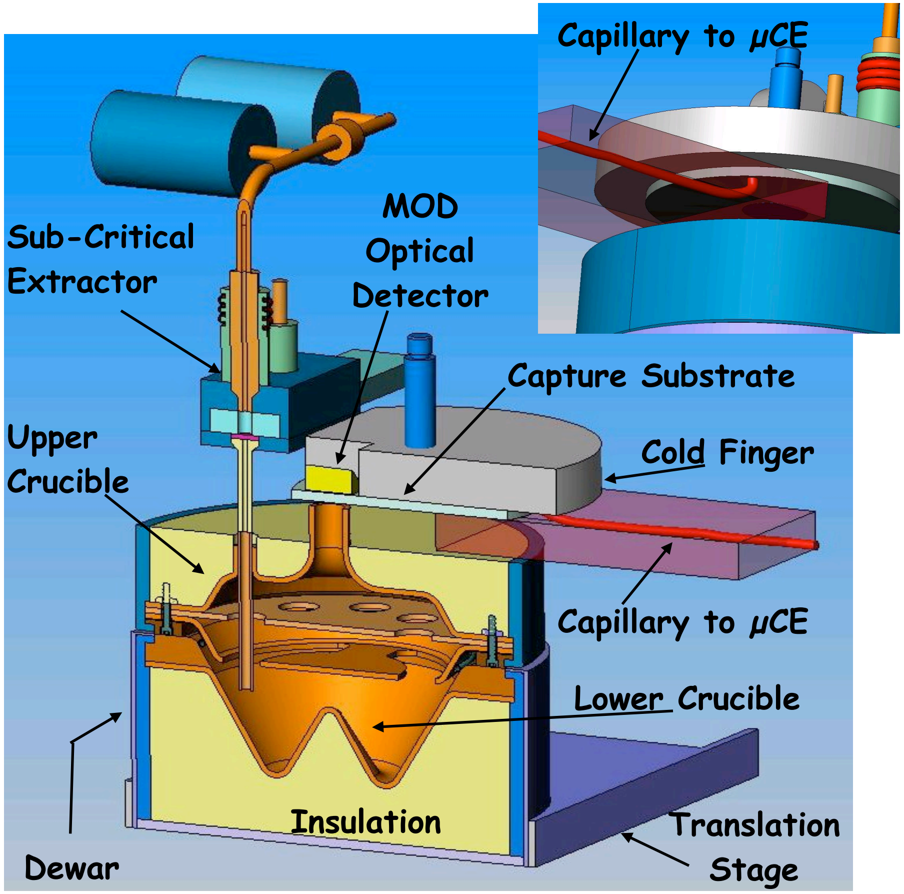


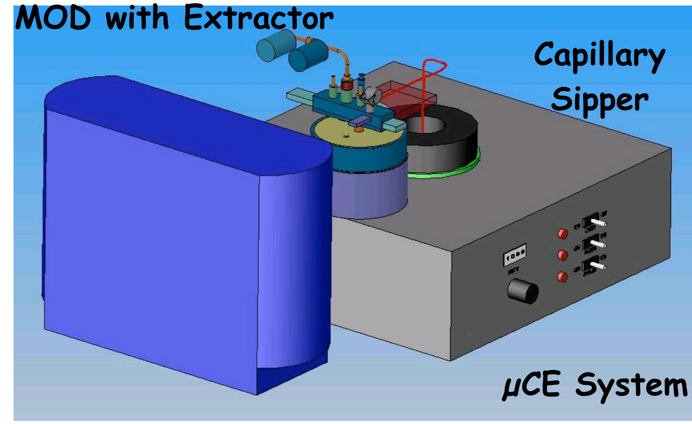
Portable extractor

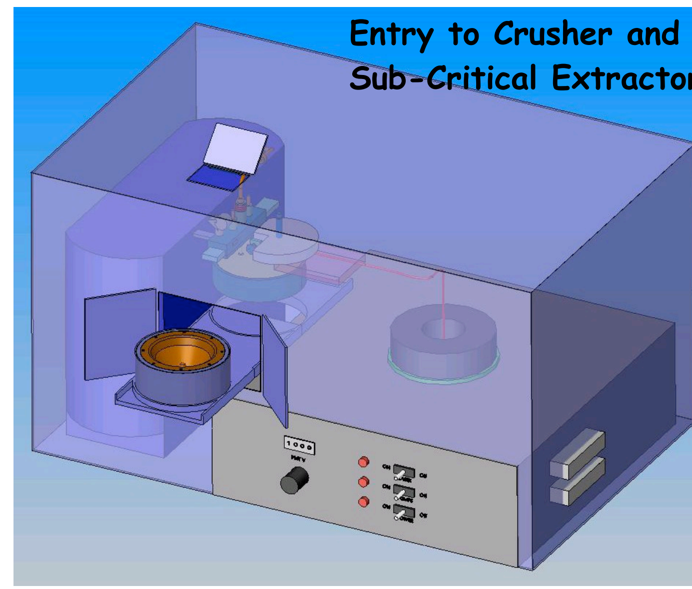
MOD/mCE and Sub-Critical Extractor Design



Integrated MOD/mCE Extractor Field Prototype





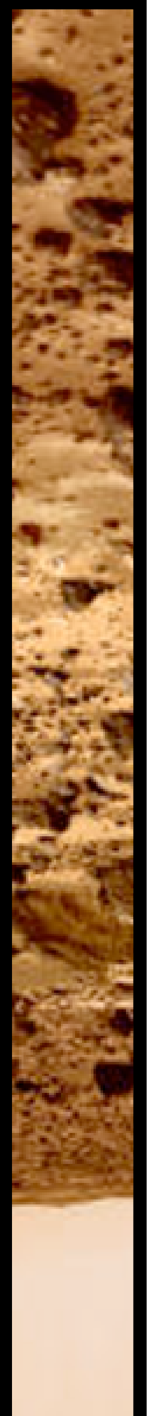


This instrument package will soon be tested at field sites su
as the Atacama desert.

Atacama Desert as Martian Soil Analog Chemical Laboratory

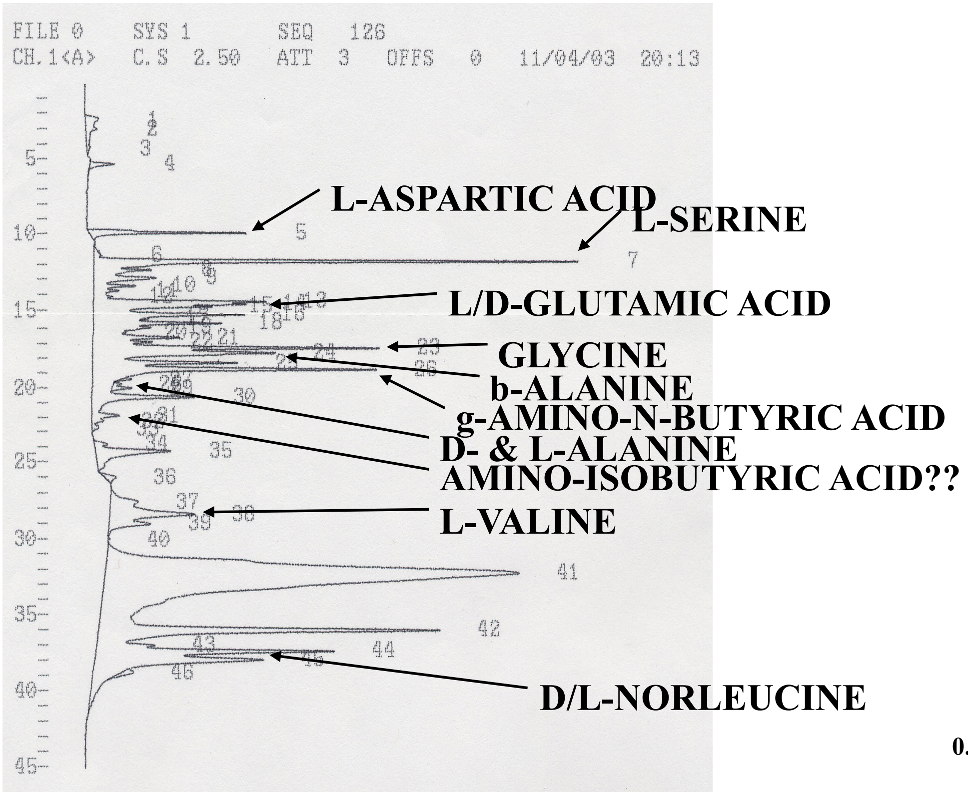
- Chilean Atacama Desert is one of the driest sites on the planet (<0.5 mm H_2O/year)
- We have extensively studied the transect from Lat 24° to 28° South at 69.5° West
- Some areas have unusual surface oxidation chemistry and organic soil concentrations at lab blank levels. Other areas show readily detected microbial and higher life forms

details in Science 302, 1018-1021 (2003)

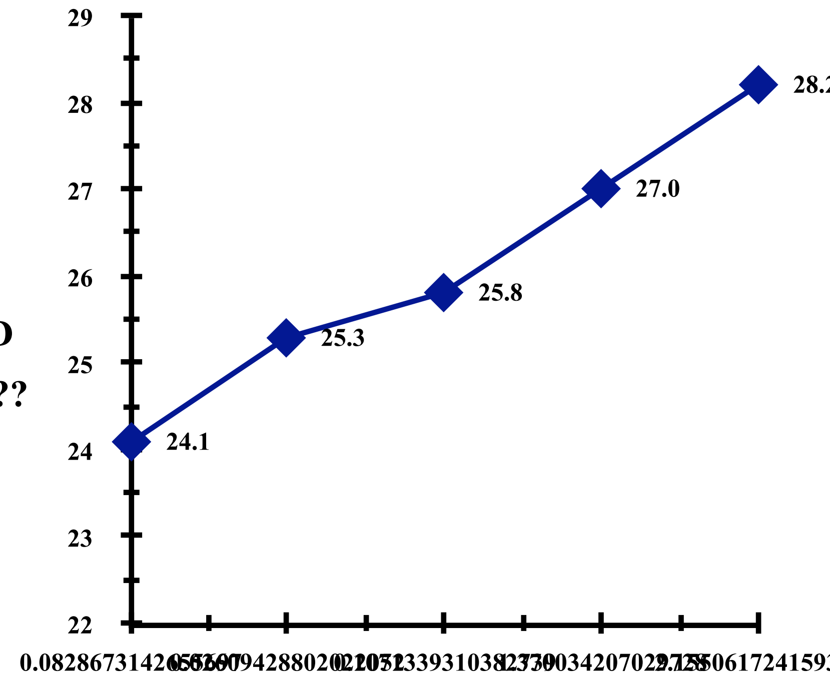


Laboratory based chiral amino acid analyses of Atacama samples

Sample AT01-22 (28.2° South)



Latitudinal Variation of Total Concentration of Amino Acids



Viking-type GCMS analyses of these same samples failed to detect the presence of amino acids, expect possible traces of ethylamine from alanine decomposition in sample AT01-22 (Navarro-González, pers. comm).

MOD Field Prototype

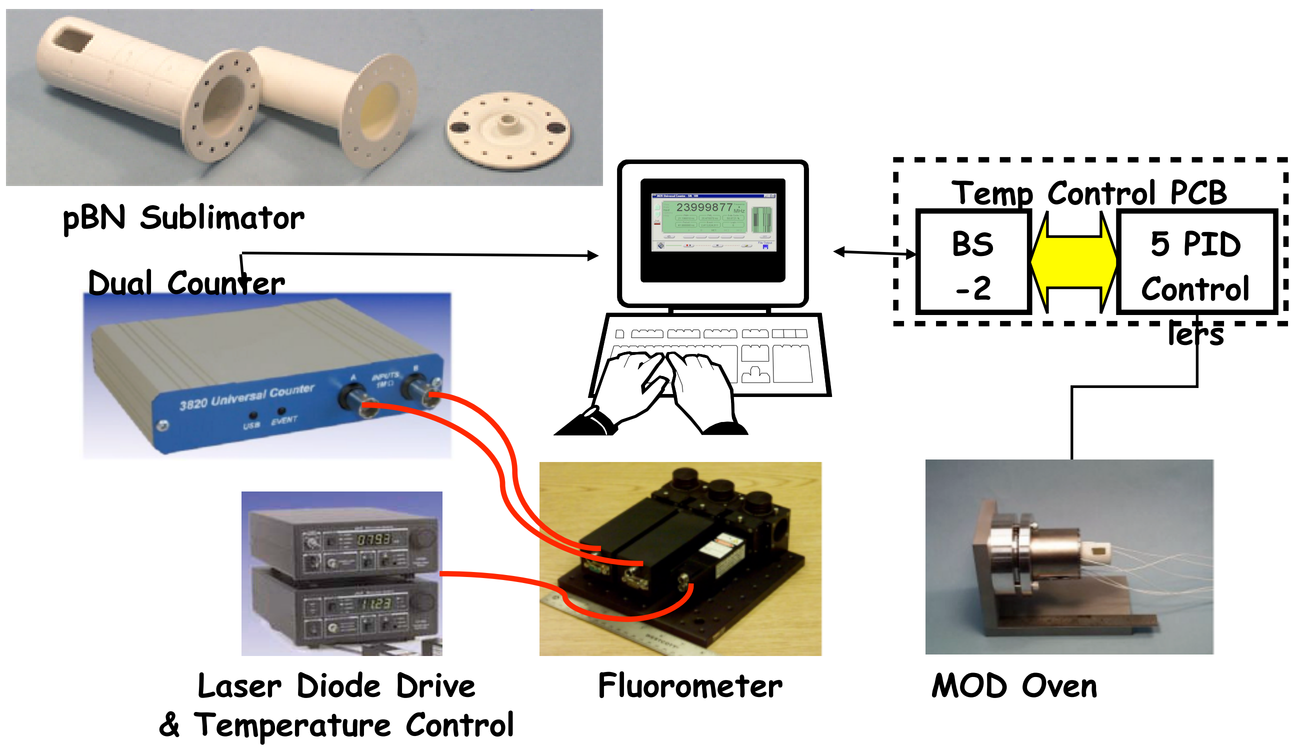
- **Science**

– in situ detection of key organic compounds, amino acids and polycyclic aromatic hydrocarbons (PAHs), at a sensitivity of roughly 100 -1000x better than Viking.

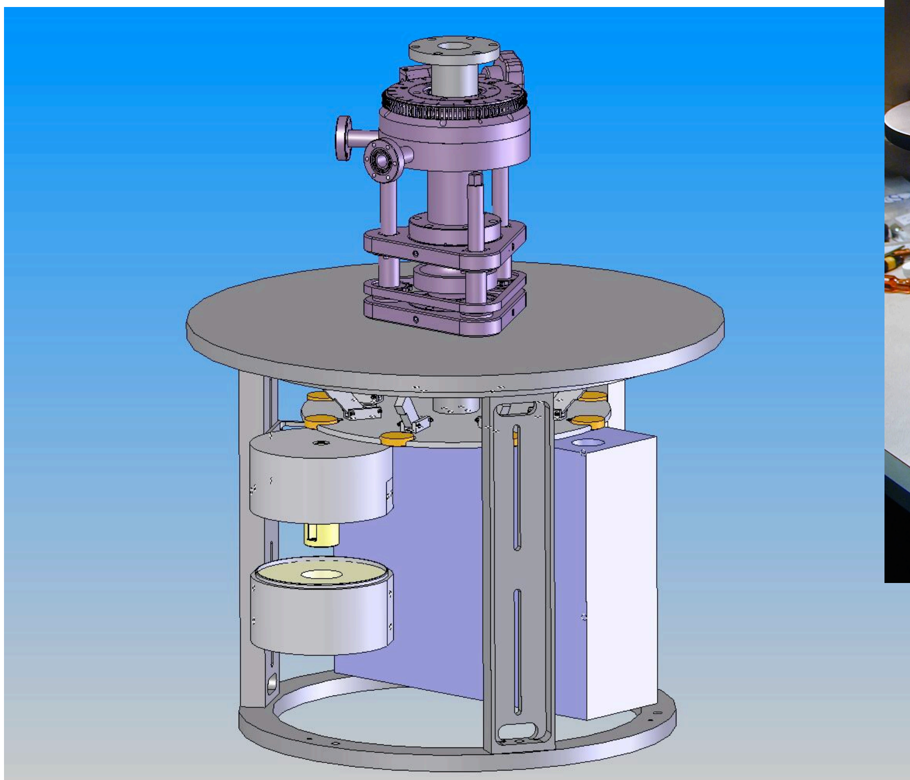
- **Approach**

– Crushed rocks and soil are heated to sublime target molecules

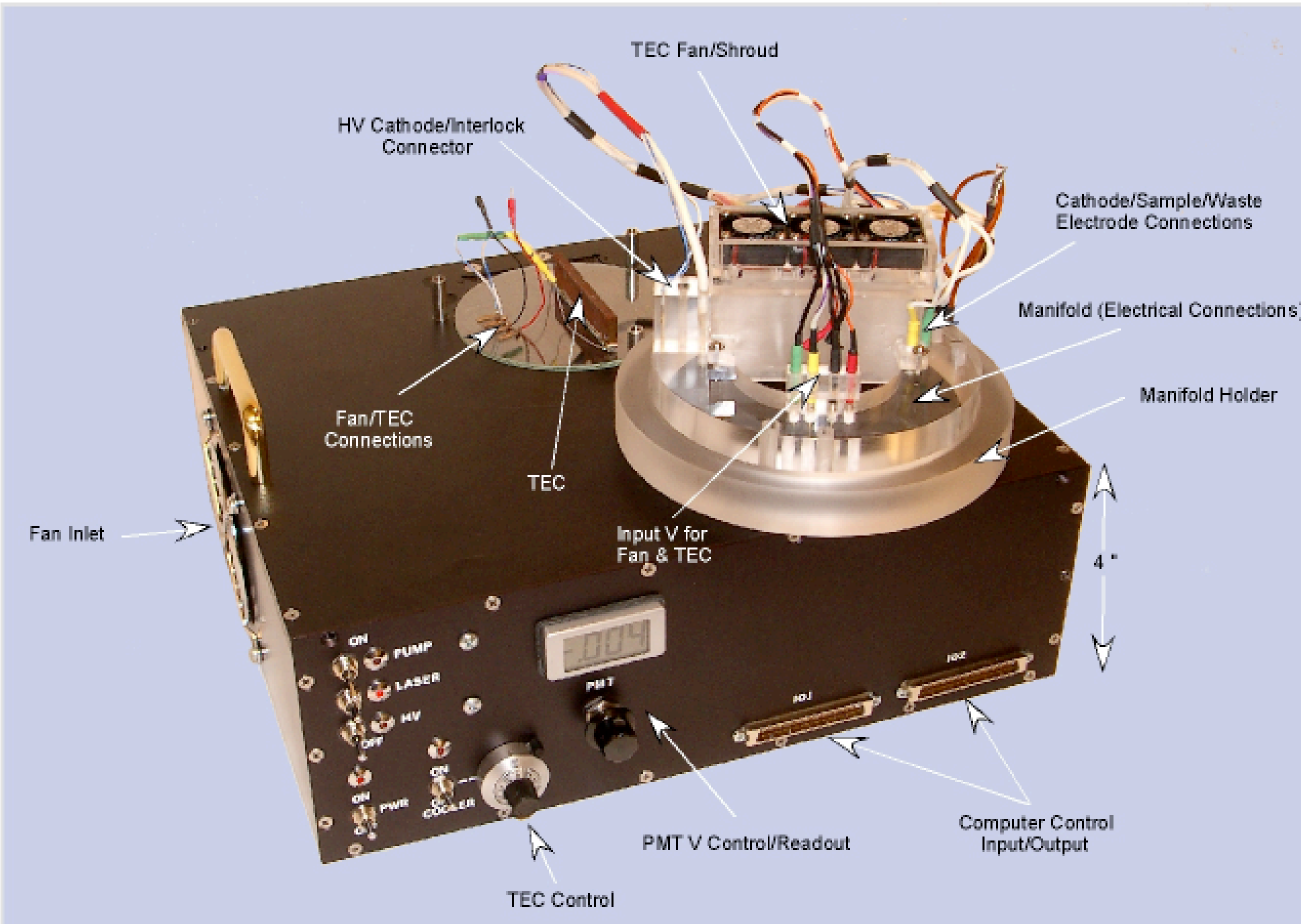
– Amino acids and PAHs are condensed on a cold finger and measured by UV fluorometer



Field Test Version of MOD with Integrated Fluorometer



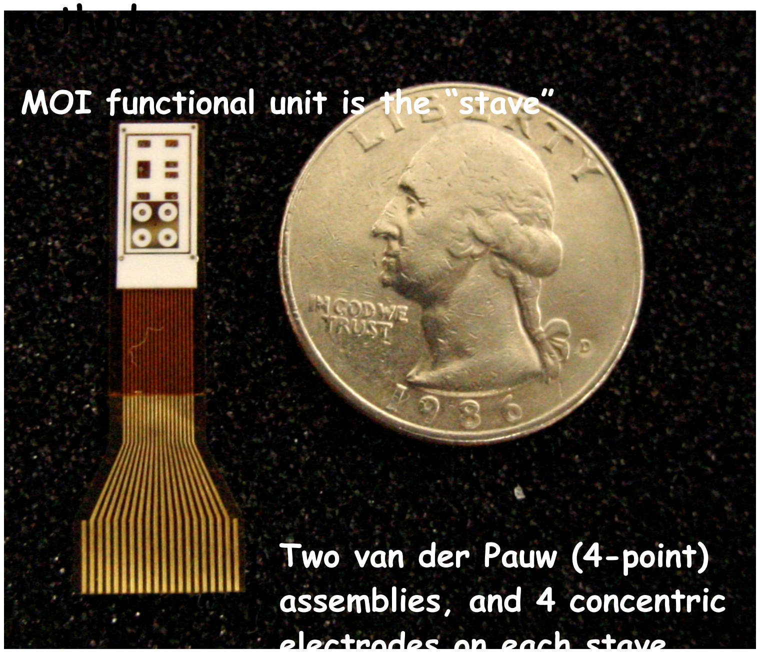
Micro-fabricated Amino Acid Analyzer

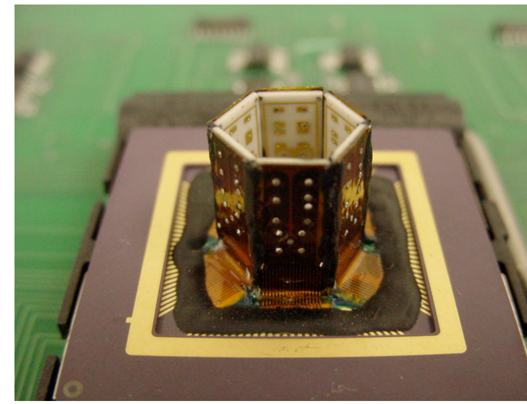


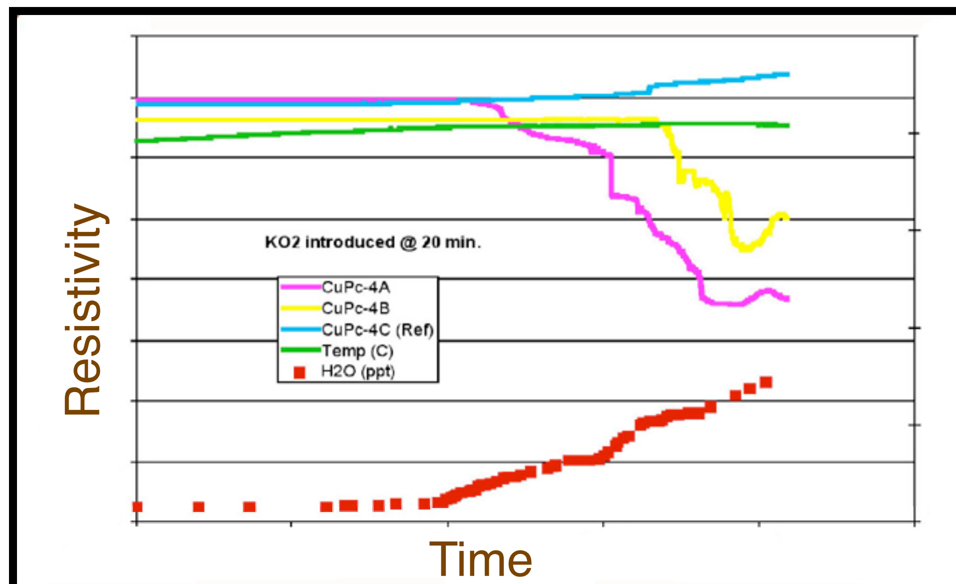
- Portable analyzer for determination of amino acid composition and chirality
- Self-contained with all high voltage, laser, detector and pumping power supplies as well as microfluidic and CE interface control
- Ready interchange of CE modules
- Sipper/ capillary interface to MOD/ fluorescamine module
- Box measures 4 x 10 x 10 inches

The MOI Instrument

- MOI is designed to monitor the reactions of Martian soils in intimate contact with well-characterized reference reactants.
- The chemical state of the films is monitored by measuring their electrical resistivity, via a chemiresistor transducer
- 200 ppm KO_2, mixed with Mars soil analog is easily detected.
- Data analysis by pattern recognition and chemometric







AstroBioLab Status

- MOD/mCE, extraction system and MOI instruments make up an integrated package ready for testing in the field
- MOD and sample extraction front end processing is compact, mature and highly flexible (oven technology supports 1300°C)
- MOD/mCE could be integrated with the pyrolysis GCMS instruments for enhanced compound identification.

Technology Readiness Level

- MOD and MOI were developed to TRL 4 in previous program funding
- Integrated MOD/mCE, extraction system and MOI reach TRL 6 following ASTEP funding and Atacama desert field trials
- Integration with MSL sampling systems and possible interface with pyrolysis GCMS (will entail new engineering issues)

Conclusions

- **Major advances have been made in basic MOD, extraction system and mCE designs and interface. AstoBioLab is ready for deployment now!**
- **Major experimental insights into sample handling, sample extraction and *in situ* instrumentation integration.**
- **Science evolution and TRL enhanced with field campaigns**
- **Strong synergism between organic biomarker detection and oxidation sensor analysis**
- **Atacama is an excellent (challenging) site to refine detection technologies and performance characteristics for the AstroBioLab instrument package.**